

CLAIMS

[0237] We claim:

1. A method for obtaining a transgenic monocotyledon plant containing a gene of interest (i) that is free of foreign ancillary sequence comprising:
 - (a) contacting a plant or a cell of a plant that lacks an active transposase, with a transformation vector comprising:
 - (1) a first expression cassette comprising a gene of interest (i) that is not operably linked to mobilizable sequences of a transposon; and
 - (2) a second expression cassette comprising a nucleotide sequence encoding a selection marker (ii) that is operably linked to the mobilizable sequences of a transposon, wherein said nucleotide sequence encoding a selection marker (ii) is operably linked to a plant expression control sequence,to obtain primary transformants;
 - (b) growing the primary transformants under selective conditions to obtain at least one transformed parental plant or plant cell having the selection marker gene (ii);
 - (c) crossing the selected transformed parental plant with a second parental plant, said second plant having within its genome a gene encoding an endogenous active transposase, wherein the transposase gene interrupts expression of a gene encoding a phenotypic marker for excision (iii), such that an F1 generation is obtained;

(d) selecting a plant or cell from the F1 generation having the gene of interest (i) but lacking the selection marker gene (ii); and

(e) regenerating a plant from the plant or cell selected in (d),

such that a transgenic monocotyledon plant containing a gene of interest (i) that is free of foreign ancillary sequence is produced.

2. The method of claim 1, wherein the selection marker gene (ii) is selected from the group consisting of an antibiotic resistance gene, a herbicide resistance gene, and a phenotypic marker gene.
3. The method of claim 1, wherein the selection marker gene (ii) is selected from the group consisting of an nptII gene and a bar gene.
4. The method of claim 1, wherein the second expression cassette further comprises a nucleotide sequence encoding a reporter protein, wherein the reporter protein is nondestructively detectable.
5. The method of claim 4, wherein the reporter protein is a green fluorescent protein.
6. The method of claim 1, wherein the endogenous active transposase is Activator and the mobilizable sequences of a transposon are Dissociation elements.
7. The method of claim 1, wherein the progeny plants or cells in step (d) are selected from the group consisting of F1 plants, F2 plants, and calluses.
8. The method of claim 1, wherein the transgenic monocotyledon plant is a maize plant.

9. The method of claim 8, wherein the transgenic monocotyledon plant belongs to the A188 line.
10. The method of claim 8, wherein said second plant in step (c) belongs to an R-nj::Ac line.
11. The method of claim 10, wherein the R-nj::Ac line is selected from the group consisting of the W22/R-nj As line, the W22/R-nj Ag line, and the A188/R-nj::Ac line.
12. The method of claim 1, wherein the selection of the progeny plant or cells in step (d) comprises:
- selecting variegated F1 seeds;
 - selecting F1 plants displaying somatic excision of the selection marker gene (ii);
 - selecting F1 plants displaying germinal excision of the selection marker gene (ii);
 - obtaining F2 plant sowing based on these events.
13. The method of claim 1, wherein the selection of the progeny plant or cells in step (d) comprises:
- producing calluses from immature F1 embryos,
 - visually selecting the calluses containing the T-DNA and the selection marker (ii),
 - multiplying calluses and selecting sectors of excision of the selection marker gene (ii),
 - regenerating F1 plants from the selected sectors of excision.

14. The method as claimed in claim 13, wherein regenerating plants from the progeny plants or cells selected in (d) comprises culturing selected calluses of immature embryos of F1 ears under conditions that allow regeneration of plants.
15. A transgenic monocotyledon plant, or a part of a plant, containing a gene of interest (i) that is free of foreign ancillary sequence obtained by the method of claim 1.
16. A hybrid monocotyledon plant, characterized in that it is obtained by crossing a plant of claim 15.
17. The plant or part of a plant of claim 15, wherein the plant is maize.
18. A vector comprising:
 - (1) a first expression cassette comprising a gene of interest (i) that is not operably linked to mobilizable sequences of a transposon; and
 - (2) a second expression cassette comprising a nucleotide sequence encoding a selection marker (ii) that is operably linked to the mobilizable sequences of a transposon, wherein said nucleotide sequence encoding a selection marker (ii) is operably linked to a plant expression control sequence.
19. A host cell comprising the vector of claim 18.
20. A plant cell, or a clone of such a cell comprising:
 - (1) a first expression cassette comprising a gene of interest (i) that is not operably linked to mobilizable sequences of a transposon; and

(2) a second expression cassette comprising a nucleotide sequence encoding a selection marker (ii) that is operably linked to the mobilizable sequences of a transposon, wherein said nucleotide sequence encoding a selection marker (ii) is operably linked to a plant expression control sequence.

21. A method for selecting callus cells exhibiting an excision of the selection marker (ii) for obtaining transgenic plants containing a gene of interest (i) that is free of foreign ancillary sequence, comprising the steps:

- producing calluses from immature F1 embryos,
- visually selecting the calluses containing the T-DNA and the selection marker (ii),
- multiplying calluses and selecting sectors displaying excision of the selection marker (ii).